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Nitric oxide functions as a signal in ultraviolet-B induced inhibition of pea stems elongation

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Abstract

The effects of ultraviolet-B (UV-B) radiation on stem elongation growth, xyloglucan-degrading activity, the rate of nitric oxide (NO) release and nitric oxide synthase (NOS) activity were evaluated in stems of pea (*Pisum sativum* L.) seedlings during a 5 days growth period. UV-B radiation significantly induced NOS activity and promoted NO release subsequently inhibited xyloglucan-degrading activity which led to the inhibition of pea stems elongation. Similarly, exogenous NO donation to the rhizospheric zone of pea seedlings imitated the responses of stems to UV-B radiation. NOS inhibitor (LNNA) and NO scavenger (PTIO) had the opposite responses to UV-B radiation: decrease NO release, increase xyloglucan-degrading activity and stems elongation. The results indicated that reduction of stems length caused by UV-B radiation was possibly achieved through modification of the mechanical properties of cell wall polysaccharides, which was probably mediated by the change of xyloglucan-degrading activities in cell walls and NO might be as a signal regulating the xyloglucan-degrading activities in cell walls. In addition, the difference of NOS origin between graminaceous plants and dicots was discussed.

Keywords: UV-B radiation; Nitric oxide; NOS activity; Xyloglucan-degrading activity; Cell wall; *Pisum sativum*

1. Introduction

Depletion of the stratospheric ozone layer causes an increase in solar ultraviolet-B (UV-B, 280–320 nm) radiation reaching the Earth's surface. Enhanced UV-B radiation will have adverse effects on biological processes, owing to its high energy level [1]. The impacts of UV-B radiation on growth, development and metabolism of plants have been studied widely [2–5]. Morphogenetic changes are concerned in effects of enhanced UV-B radiation in several plants, such as inhibition of tomato seedling hypocotyl [6], shorten internodes of wheat [7], reduction in the plant height [7] and decrease in the leaf length and area of rice [8]. Since cell elongation is regulated by changing in the mechanical properties of cell walls, which can be attributed to modification of the chemical properties of the cell wall polysaccharides mediated by certain wall enzymes [9,10], it is expected that the modification of structure and metabolism of their cell wall compositions are involved in UV-B induced growth inhibition in plants. Morphological responses to UV-B radiation have been investigated extensively. However, little is know about the relationship between growth inhibition and the changes in the mechanical properties of cell walls mediated by wall enzymes.

Nitric oxide (NO) is a widespread intracellular and intercellular signal molecule that is involved in many physiological processes in animals, such as vasorelaxation, platelet inhibition, neurotransmission, cytotoxicity and immunoregulation [11]. NO has also been proved to be a signal

Abbreviations: CK, control; FW, fresh weight; LNNA, N^{ω}-nitro-L-arginine; NO, nitric oxide; NOS, nitric oxide synthase; PTIO, 2-phenyl-4,4,5,5-tetramethyl-imidazoline-l-oxyl-3-oxide; SNP, sodium nitroprusside; UV-B radiation, ultraviolet-B radiation; $UV\text{-}B_{BE}$ radiation, biologically effective UV-B radiation

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molecule playing important roles in diverse physiological processes in plants, including growth and development [12,13], hormones modulation [14] and biotic/abiotic stresses [15,16]. In animal systems, most of the NO is synthesized by nitric oxide synthase (NOS) [17]. Immunological analysis suggests that NOS-like proteins probably localize in the cytoplasm of maize [18], peroxisomes of pea and olive leaves [19,20] and sunflower hypocotyls [20]. Recently, a nitric oxide synthase gene (*AtNOS1*) in Arabidopsis plants regulating growth and hormonal signaling was identified [21]. This demonstrates that NOS enzymes exist in plants. And this NOS activity can be inhibited by NOS inhibitor of mammalian [21].

UV-B induced up-regulation of chalcone synthase gene (*CHS*) involves NO originated from NOS activity [22]. NO also play an important role in UV-B-induced ethylene synthesis [23]. NO may be the second messenger of UV-B induced growth inhibition, and the next signal molecule following NO is likely to exo- and endo-β-D-glucanase in cell walls of maize [24,25]. Since the responses to UV-B radiation in different taxa and different cultivars can be dissimilar or even reverse [26,27] and the compositions of cell wall polysaccharides of dicots differ from those of graminaceous plants [28], the signaling cascade mechanisms of UV-B-induced growth inhibition in dicots and graminaceous plants are likely to be diverse. The signaling cascade mechanisms of UV-B-induced growth inhibition had been investigated in maize, a graminaceous species [24,25]. However, the potential signaling pathways of UV-B-induced growth inhibition in dicots have not been clarified. It is essential to study whether or not interaction of NO with wall enzymes of dicots can affect cell elongation and the chemical properties of cell walls to understand the signaling cascade mechanisms of UV-B-induced growth inhibition. In this paper, we measured the effects of NO on the activity of xyloglucan-degradation enzymes and stem elongation of pea seedlings. The objective of the present study was to evaluate the possible signaling pathways in dicots under UV-B radiation and functions of NO as a signal of UV-B radiation.

2. Materials and methods

2.1. Plants material

Seeds of pea (*Pisum sativum* L. No. 8711-2, from Gansu Academy of Agricultural Sciences) were pre-germinated for 24 h in moistened plates at 25 °C and sown in plastic plots filled with perlite. Seedlings were grown at 25 °C with 12 h photoperiod under the 100 μM photo $m^{-2} s^{-1}$ (400–700 nm) in a controlled environmental growth chamber. The lost water was supplemented every day. When the second trifoliate leaf was completely developed, half of the selected uniform seedlings were transferred to a UV-B chamber supplemented with UV-B radiation. At the same time, the other half of the selected uniform seedlings were continue to grow for a further 5 day for a group of controls without UV-B irradiation. The plants were irrigated by Hoagland solution or a few chemicals added into the Hoagland solution. Each of the three pots was regarded as a group of treatments and all experiments were independently repeated at least three times. Stem samples were randomly taken from uniform plants in different parts of the pots. Fresh tissues were directly used for NO assay, or were froze with liquid N_2 and stored at −70 °C for enzyme analysis.

2.2. UV-B radiation treatment

Enhanced UV-B radiation was generated with filtered Qin brand (Baoji Lamp Factory, China) 30 W fluorescence sunlamps (290–320 nm) following the procedure outlined in ref. [29]. The lamps were suspended above and perpendicular to the plastic plots and filtered with 0.13 mm thick cellulose diacetate (transmission down to 290 nm) for UV-B irradiance. Cellulose diacetate filters were pre-solarized. The desired irradiation was obtained by changing the distance between the lamps and the top of seedlings. The spectral irradiance from the lamps was determined with an Optronic Model 742 (Optronic Labs., Orlando, FL, USA) spectroradiometer and weighted with the generalized plant response action spectrum [30] and normalized at 300 nm to obtain the desired level of biologically effective UV-B radiation ($UV\text{-}B_{BE}$). The levels of UV-B irradiation were 4.8 kJ m^{-2} per day.

2.3. Preparation of xyloglucans

Xyloglucans were prepared from pea epicotyls as described previously [31]. Fresh epicotyls (5–6 cm) were collected and were boiled in methanol for 10 min, then were homogenized. The insoluble fraction was washed twice each with cold water, acetone, a mixture of methanol and chloroform (1:1, v/v) and ethanol. The cell wall fraction was digested in 10 units/ml α-amylase (EC 3.2.1.1, from porcine pancreas, Sigma) in 50 mM sodium acetate buffer (pH 6.5) for 3 h at 37 °C. Then pectic substances were extracted from the cell wall materials three times (15 min each) with 50 mM EDTA (pH 6.8) at 95 °C. Then cell wall material was extracted three times (each 24 h) with 24% (w/v) KOH containing 0.02% $NaBH_4$ at 25 °C. The extracts were neutralized with acetic acid, dialyzed against water and lyophilized. The lyophilized extract was dissolved in 0.1 M NaOH, and applied to a column of Sepharose CL-4B (1.6 cm × 80 cm), equilibrate with 0.1 M NaOH. The eluate passing through the column was collected. The xyloglucans was calculated by a calibration curve obtained with authentic dextrans (500, 40 and 10 kDa, Sigma). The xyloglucan of 400–600 kDa were selected.

2.4. Chemical and UV-B plus chemical combined treatments of pea seedlings

Chemical treatments were based on the method of Zhang et al. [24]. The SNP (NO donor), LNNA (NOS inhibitor) and PTIO (NO scavenger) were applied in vivo and were absorbed through the roots [32]. The seedlings were cultivated in perlite